

**AMENDMENTS TO THE CLAIMS:**

This listing of claims will replace prior versions and listings of claims in the application:

**Listing of claims:**

Claims 33-35, 39, 69-71 and 75 have been cancelled. Claims 86 to 114 are new. Claims 30-32, 36-38, 40, 45-47, 49, 51 and 66 have been amended as follows: Underlines indicate insertions and ~~strikeouts~~ indicate deletions.

30. (Currently amended) A soluble ~~proteic~~ fragment of a subtilisin-kexin isoenzyme named SKI-1 which has the amino acid sequence defined by amino acids 187 to 996 of any one SEQ ID NOs: 2, 4 and 6, or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 187 to 996 and a variant thereof, which is enzymatically active.
31. (Currently amended) A ~~proteic~~ fragment of a subtilisin-kexin isoenzyme named SKI-1, which has the amino acid sequence defined by amino acids ~~48-17~~ to 137 of any one of SEQ ID NOs: 2, 4 and 6, or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 137 or a part thereof and a variant thereof, which is capable of binding with amino acids ~~48-17~~ to 1052 of SKI-1 ~~in whole or in part~~.
32. (Currently amended) The ~~proteic~~ fragment of claim 31, wherein said part has a molecular weight of about 14 kDa and forms a tight complex with the soluble fragment of SKI-1.
33. (cancelled)
34. (cancelled)
35. (cancelled)
36. (Currently amended) An isolated nucleic acid encoding a ~~protein~~ fragment as defined in claim 30.
37. (Currently amended) An isolated nucleic acid encoding a ~~proteic~~ fragment as defined in claim 31.
38. (Currently amended) An isolated nucleic acid encoding a ~~proteic~~ fragment as defined in claim 32.
39. (cancelled).

40. (Previously added) A recombinant vector comprising the nucleic acid defined in claim 36.
41. (Previously added) The recombinant vector of claim 40, which is an expression vector.
42. (Previously added) The recombinant vector of claim 41, which comprises a promoter expressible in a target cell wherein expression of said nucleic acid is desirable.
43. (Previously added) The recombinant vector of claim 42, which comprises an inducible promoter.
44. (Previously added) A recombinant host cell comprising the recombinant vector defined in claim 40.
45. (Currently amended) A method of producing a ~~proteic~~ fragment of SKI-1 enzyme, which comprises the steps of:  
culturing a recombinant host cell expressing a nucleic acid as defined in claim 36 in a cell growth and expression-supportive culture medium; and  
recovering said ~~proteic~~ fragment of SKI-1 in the culture medium.
46. (Currently amended) A method for cleaving a substrate for SKI-1 enzyme, which comprises the step of:  
a) ~~contacting said substrate with a SKI-1 enzyme which has 1) an amino acid sequence defined by amino acids 18 to 1052 of SEQ ID Nos: 2, 4, 6 and an active variant thereof, or 2) a SKI-1 soluble fragment of a subtilisin-kexin isoenzyme named SKI-1 which has the amino acid sequence defined by amino acids 187-996 of any one of SEQ ID NOs 2, 4 and 6;~~ or 2) an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 187 to 996 and a variant thereof, which is enzymatically active;  
or 3) a catalytic part of a1) or b2); or 4) a complex as defined in claim 32, for a time sufficient and in conditions adequate for such cleavage to occur,  
with the proviso that said substrate is not a sterol-regulatory element-binding protein (SREBP) and is not SKI-1.
47. (Currently amended) A method for producing a protein or a peptide from a ~~proteic~~ precursor which is an enzymatic substrate for SKI-1 enzyme, which comprises the steps of:  
a) ~~contacting said ~~proteic~~ precursor with a SKI-1 enzyme which has 1) an amino acid sequence defined by amino acids 18 to 1052 of SEQ ID Nos: 2, 4, 6 and an active variant thereof, or 2) a SKI-1 soluble fragment of a subtilisin-kexin isoenzyme named SKI-1 which has the amino acid sequence defined by amino acids 187-996 of any one of SEQ ID NOs 2, 4 and 6;~~ or 2) an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 187 to 996 and a variant thereof, which is enzymatically active;  
or 3) a catalytic part of a1) or b2); or 4) a complex as defined in claim 32, for a time sufficient and in conditions adequate for such cleavage to occur; and

b) recovering said protein or peptide;  
with the proviso that said substrate is not a sterol-regulatory element-binding protein (SREBP) and is not SKI-1.

48. (Previously added) The method of claim 47, which takes place in a cell or in the presence of a cellular population and wherein step a) comprises the step of transfecting a cell with a nucleic acid expressing said SKI-1 enzyme.

49. (Currently amended) The method of claim 48, wherein said cell expresses said ~~proteic~~ precursor or is transfected with a nucleic acid expressing said ~~proteic~~ precursor.

50. (withdrawn) A method of inhibiting the activity of a subtilisin-kexin isoenzyme named SKI-1, which comprises the step of contacting SKI-1 with the inhibitor of claim 33 or isolated nucleic acid encoding the inhibitor.

51. (Currently amended) A peptide of at least 7 amino acids capable of binding to and of being cleaved by SKI-1 catalytic site, comprising the following general formula: Arg Xaa<sub>1</sub> JXaa<sub>2</sub> ↓Xaa<sub>3</sub> (Z)<sub>n</sub>O

wherein Xaa<sub>1, 2, 3</sub> and Z are any amino acid  
J is an alkyl or aromatic hydrophobic amino acid  
n is 1, 2 or 3  
O is an acidic amino acid,

with the proviso that ~~the sequence said peptide~~ does not comprise the sequence Lys-Arg-Phe-Val-Phe-Asn-Lys-Ile-Glu and with the proviso that said peptide is not a sterol-regulatory element-binding protein (SREBP) or a part thereof or SKI-1 or a part thereof.

52. (Previously added) A peptide as defined in claim 51, wherein Xaa<sub>2</sub> is Lys, Leu, Phe or Thr.

53. (Previously added) A peptide as defined in claim 52 which has the sequence: H<sub>2</sub>N-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-COOH.

54. (Previously added) A peptide as defined in claim 51 which is labelled.

55. (Previously added) A peptide as defined in claim 54 which is fluorogenic.

56. (Previously added) A peptide as defined in claim 55 which is Abz-Val-Phe-Arg-Ser-Leu-Lys-Tyr-Ala-Glu-Ser-Asp-Tyr(NO<sub>2</sub>),  
wherein Abz is orthoaminobenzoic acid, and  
Tyr(NO<sub>2</sub>) is 3-nitrotyrosine.

57-58. (Cancelled)

59. (Previously added) A method for screening for a polypeptide that has the activity of a subtilisin-kexin isoenzyme named SKI-1, the method comprising the steps of:

contacting the peptide of claim 51 to a test polypeptide under conditions that allow cleavage of the peptide by a SKI-1; and

detecting the cleavage of the peptide wherein the presence of the cleavage indicates that the polypeptide has SKI-1 activity.

60. (Previously added) A method for monitoring the activity of a subtilisin-kexin isoenzyme named SKI-1 comprising the steps of:

contacting a sample having or suspected of having SKI-1 activity with the peptide of claim 51; and

monitoring the cleavage of the peptide.

61. (withdrawn) A method for screening inhibitors or substrates of a subtilisin-kexin isoenzyme named SKI-1 comprising the steps of:

contacting the protein which has SKI-1 activity with the peptide of claim 51 in the presence of a test compound under conditions that allow cleavage of the peptide by the protein with SKI-1 activity;

determining the cleavage of the peptide; and

comparing the cleavage of the peptide with that of a control group in which the protein with SKI-1 activity is contacted with the peptide of claim 51 in the absence of the test compound under the same conditions wherein a lower than control cleavage rate indicates that the test compound is an inhibitor or substrate of SKI-1.

62. (withdrawn) A method for treating a disease related to an overexpression of a SKI-1 or a SKI-1 substrate in a human or non-human animal, the method comprising the step of:

administering to the human or non human animal an inhibitor of the activity of SKI-1 in an amount sufficient to inhibit the activity.

63. (withdrawn) The method of claim 62, wherein said disease is associated with any one of hypercholesterolemia, high levels of fatty acids, lipids or farnesyl pyrophosphate, liver steatosis, Ras-dependent cancer, restenosis and amyloid protein formation.

64. (withdrawn) The method of claim 62, wherein said inhibitor is defined in claim 31.

65. (Previously added) A composition comprising a SKI-1 fragment as defined in claim 30.

66. (Currently amended) A method for cleaving a ~~proteic~~ precursor which is SKI-1 substrate, the method comprising the steps of:

providing a SKI-1 enzyme as encoded by a nucleic acid having a nucleotide sequence of nucleotides 469 to 3573 of SEQ ID NO: 1, nucleotides 59 to 3163 of SEQ ID NO: 3 or nucleotides 548 to 3652 of SEQ ID NO: 5, a catalytic part of SKI-1 enzyme that is unique to SKI-1 enzyme and encoded by the corresponding sequence of SEQ ID NOs: 1, 3 or 5, or an active variant of the SKI-1 enzyme or the catalytic part, wherein the nucleotide sequence that encodes the variant shares at least 70% homology with a nucleotide sequence

on SEQ ID NOs: 1, 3 or 5 and hybridizes to SEQ ID NOs: 1, 3 or 5 under stringent hybridization conditions, and

contacting the ~~proteie~~ precursor with the SKI-1 enzyme, the catalytic part of SKI-1, or the active variant of the SKI-1 enzyme or the catalytic part under conditions that allow the cleavage of the ~~proteie~~ precursor, with the proviso that said substrate is not a sterol-regulatory element-binding protein (SREBP).

67.(Previously added) A composition comprising a SKI-1 fragment as defined in claim 31.

68.(Previously added) A composition comprising a SKI-1 fragment as defined in claim 32.

69.(cancelled).

70.(cancelled).

71.(cancelled).

72.(Previously added) A composition comprising a nucleic acid as defined in claim 36.

73.(Previously added) A composition comprising a nucleic acid as defined in claim 37.

74.(Previously added) A composition comprising a nucleic acid as defined in claim 38.

75-79. (Cancelled)

80.(Previously added) A composition comprising a recombinant vector as defined in claim 40.

81.(Previously added) A composition comprising a recombinant vector as defined in claim 41.

82.(Previously added) A composition comprising a recombinant vector as defined in claim 42.

83.(Previously added) A composition comprising a recombinant vector as defined in claim 43.

84.(New) A method of inhibiting SKI-1 activity comprising the step of contacting a prosegment of about 24kDa of a subtilisin-kexin isoenzyme named SKI-1, with SKI-1.

85.(New) A method as recited in claim 84, wherein the prosegment is a native prosegment and has the amino acid sequence defined by amino acids 17 to 186

of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 186.

86. (New) The method of claim 84, wherein the prosegment amino acid sequence is modified to prevent further enzymatic processing in a cell expressing said prosegment.
87. The method as recited in claim 86, wherein said amino acid sequence is modified at the internal primary cleavage site to prevent the creation of a 14kDa N-terminal fragment.
88. (New) The method of claim 86, wherein the amino acid sequence is modified by amino acid substitution, deletion, rearrangement or addition.
89. (New) The method of claim 88, wherein the amino acid sequence modified is defined by amino acids 17 to 188 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 188.
90. (New) The method of claim 88, wherein the amino acid sequence modified is defined by amino acids 1 to 197 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 197.
91. (New) The method of claim 88, wherein the amino acid sequence modified is defined by amino acids 1 to 169 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 169.
92. (New) A polypeptide defined by amino acids 1 to 188 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 188.
93. (New) A polypeptide defined by amino acids 1 to 197 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 197.
94. (New) A polypeptide defined by amino acids 1 to 169 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 169.
95. (New) A polypeptide defined by amino acids 17 to 188 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 188.
96. (New) A polypeptide defined by amino acids 17 to 197 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 197.

97. (New) A polypeptide defined by amino acids 17 to 169 of SEQ ID NO: 6 or by an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 17 to 169.
98. (New) An isolated nucleic acid encoding a polypeptide as defined in claim 92.
99. (New) An isolated nucleic acid encoding a polypeptide as defined in claim 93.
100. (New) An isolated nucleic acid encoding a fragment as defined in claim 94.
101. (New) An isolated nucleic acid encoding a fragment as defined in claim 95.
102. (New) An isolated nucleic acid encoding a fragment as defined in claim 96.
103. (New) An isolated nucleic acid encoding a fragment as defined in claim 97.
104. (New) A composition comprising a polypeptide as defined in claim 92.
105. (New) A composition comprising a polypeptide as defined in claim 93.
106. (New) A composition comprising a polypeptide as defined in claim 94.
107. (New) A composition comprising a polypeptide as defined in claim 95.
108. (New) A composition comprising a polypeptide as defined in claim 96.
109. (New) A composition comprising a polypeptide as defined in claim 97.
110. (New) A method of inhibiting SKI-1 activity comprising contacting a cell with a prosegment having the amino acid sequence defined by amino acids 1 to 186 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 186 of a subtilisin-kexin isoenzyme named SKI-1, with SKI-1.
111. (New) A method of inhibiting SKI-1 activity comprising contacting a cell with a prosegment having the amino acid sequence defined by amino acids 1 to 188 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 188 of a subtilisin-kexin isoenzyme named SKI-1, with SKI-1.
112. (New) A method of inhibiting SKI-1 activity comprising contacting a cell with a prosegment having the amino acid sequence defined by amino acids 1 to 197 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 197 of a subtilisin-kexin isoenzyme named SKI-1, with SKI-1.

113. (New) A method of inhibiting SKI-1 activity comprising contacting a cell with a prosegment having the amino acid sequence defined by amino acids 1 to 169 of SEQ ID NO: 6 or an amino acid sequence from another mammalian species corresponding to the sequence of said amino acids 1 to 169 of a subtilisin-kexin isoenzyme named SKI-1, with SKI-1.